J Gastroenterol 2004; 39:927–930
DOI 10.1007/s00535-004-1424-8

Journal of Gastroenterology

Efficacy of metronidazole for the treatment of clarithromycin-resistant *Helicobacter pylori* infection in a Japanese population

Tadashi Shimoyama, Shinsaku Fukuda, Tatsuya Mikami, Michio Fukushi, and Akihiro Munakata

First Department of Internal Medicine, Hirosaki University School of Medicine, 5 Zaifu-cho, Hirosaki 036-8562, Japan

Background. Eradication of *Helicobacter pylori* has become a common treatment for several diseases. There is an increase in antibiotic-resistant strains, which causes the failure of eradication. The aim of this study was to investigate the usefulness of metronidazole for the treatment of *H. pylori* infection in patients who failed eradication therapy. ***Methods.*** Seventy *H. pylori*-positive patients who had failed eradication treatment with first-line triple therapy, which consisted of a proton pump inhibitor, amoxicillin, and clarithromycin, were enrolled into the study. Before the second-line therapy, patients underwent endoscopy to obtain *H. pylori* strains to test susceptibility to antibiotics. Lansoprazole (30 mg b.d.), amoxicillin (750 mg b.d.), and metronidazole (250 mg b.d.) were administered for 1 week, and the result was tested by ^{13}C-UBT. ***Results.*** *H. pylori* was isolated from 62 patients, and 52 of them (83.9%) were clarithromycin resistant. There was no amoxicillin- or metronidazole-resistant strain. No major adverse effects were seen, and all the patients completed the 1-week regimen. The eradication rates of lansoprazole-amoxicillin-metronidazole were 96.2% (51/53; 95% CI, 87.0%–99.5%) using both intention-to-treat analysis and per protocol analysis. ***Conclusions.*** Lansoprazole-amoxicillin-metronidazole triple therapy is an effective and promising second-line *H. pylori* eradication therapy in a north Japanese population, which has a low frequency of metronidazole resistance.

Key words: *Helicobacter pylori*, metronidazole, clarithromycin resistance

Received: July 22, 2003 / Accepted: April 6, 2004
Reprint requests to: T. Shimoyama

Introduction

The pathogenic roles of *Helicobacter pylori* infection have been implicated in several gastroduodenal diseases. Since the eradication of *H. pylori* infection was approved under the Japanese system of health insurance in November 2002,[1] eradication therapy has become more common. However, eradication therapy is not always successful. At present, only one regimen (first-line therapy) is approved by the Japanese system of health insurance; 1 week of triple therapy with a proton pomp inhibitor [PPI; one of lansoprazole (LPZ) or omeprazole (OPZ)], amoxicillin (AMOX), and clarithromycin (CLA), while the use of other antibiotics has not been approved.[1] Therefore, an increase in the number of patients who failed eradication of *H. pylori* infection has been becoming a serious problem.

The most common cause for the failure of first-line therapy in Japan is bacterial resistance to CLA.[2] The proportion of the CLA-resistant strain is increasing, and many strains acquire resistance to this antibiotic, particularly after the failure of first-line therapy. On the other hand, resistance to AMOX is very rare, and acquisition of resistance to AMOX also rarely occurs even after the failure of first-line therapy.[3] There are many eradication regimens; however, the appropriate regimen differs according to the patterns of antibiotic resistance of each population. The prevalence of the metronidazole (MNZ)-resistant strain is lower in Japan compared with other Asian countries.[4,5] A recent study demonstrated that a triple therapy consisting of rabeprazole (RPZ), AMOX, and MNZ for 1 week achieved eradication rates of 88%.[6] Thus, triple therapy with PPI, AMOX, and MNZ appears to be an effective regimen to eradicate *H. pylori* infection in Japan. However, there are regional differences in the patterns of antibiotic resistance in Japan.[7] Furthermore, the efficacy of triple therapies of MNZ and AMOX with

other PPIs has not been fully investigated in patients who had failed first-line therapy.

The aims of this study were to investigate the antibiotic resistance of *H. pylori* after first-line therapy and to examine the efficacy of triple therapy consisting of LPZ, AMOX, and MNZ as the second-line therapy in a population of North Japan.

Materials and methods

Patients and protocols

A total of 70 patients (44 males and 36 females; mean age, 50.3 years) who had failed first-line triple therapy, consisting of a PPI (OPZ or LPZ), AMOX, and CLA, were enrolled into the study during April 2002 and May 2003. Before the second-line therapy, patients underwent endoscopy and gastric biopsies were obtained to isolate *H. pylori* strains to test susceptibility to antibiotics. Patients received LPZ (30 mg b.d.), AMOX (750 mg b.d.), and MNZ (250 mg b.d.) for 1 week if they had agreed to receive the treatment. Patients who were receiving nonsteroidal antiinflammatory drugs (NSAIDs) or other antibiotics were excluded. Finally, 53 of 70 patients received the second-line therapy. There were 30 males and 23 females; their mean age was 50.8 ± 12.8 years, and 38 were smokers. The diagnosis of the 53 patients was gastric ulcer in 28, duodenal ulcer in 17, gastroduodenal ulcers in 3, chronic urticaria in 3, idiopathic trombocytopenic purpura (ITP) in 1, and there was also a patient who had undergone endoscopic mucosal resection (EMR) to gastric cancer. After finishing the treatment, patients were allowed to administer a H_2-receptor antagonist once a day if they had dyspeptic symptoms. PPIs and other antiulcer agents, which might affect the viability of *H. pylori* or urease activity, were not used after finishing the treatment. ^{13}C-urea breath test was performed at least 6 weeks after finishing the treatment. All subjects provided written informed consent before their endoscopy, and this study was approved by the ethics committee of Hirosaki University.

H. pylori *culture and determination of minimal inhibitory concentration*

Biopsy specimens were inoculated onto Skirrow blood agar and cultured for 3–5 days at 37°C at high humidity under strict aerobic conditions (N_2 80%, CO_2, 15%, O_2 5%). The bacteria were identified as *H. pylori* by colonial morphology and positive oxidase, catalase, and urease reactions. The strains were suspended in 1 ml phosphate-buffered saline (pH 7.6) for DNA preparation. The minimal inhibitory concentration (MIC) of isolated *H. pylori* strains to AMOX, CLA, and MNZ was determined by an agar dilution method. When the MIC of AMOX, CLA, and MNZ was 0.5, 1.0, and 16.0 μg/ml or higher, the strain was assessed as resistant.

Results

Resistance to CLA was observed in 83.9% of isolated strains (52/62) after the failure of first-line therapy (Table 1). However, no strains were resistant to both AMOX and MNZ (3 patients were infected with strains of low susceptibility to MNZ: MIC was 8 μg/ml). No major adverse effects were seen, and all 53 patients completed the 1-week regimen. The eradication rate was estimated as 96.2% [51/53; 95% confidence interval (CI), 87.0%–99.5%] using both intention-to-treat analysis and per protocol analysis (Table 2). In the 40 patients who were infected with a CLA-resistant strain, eradication was successful in 39 patients (97.5%). Eradication was successful in all 3 patients who were infected with strains of low susceptibility to MNZ (these strains were also CLA resistant). Two patients who failed eradication were an 18-year-old man with ITP and a 49-year-old man with gastroduodenal ulcers.

Discussion

Since the Japanese system of health insurance approved eradication therapy for *H. pylori*, the number of

Table 1. In vitro results of susceptibility to antibiotics in 62 strains isolated from patients who failed first-line therapy (number of patients)

	Sensitive	Low sensitivity	Resistant
Amoxicillin	62	0	0
Clarithromycin	10	0	52
Metronidazole	59	3	0

Helicobacter pylori strains were determined as sensitive when minimal inhibitory concentrations (MICs) to amoxicillin, clarithromycin, and metronidazole were <0.25, <0.5, and <8, respectively; stains with MICs larger than 0.5 (amoxicillin), 1.0 (clarithromycin), and 16 (metronidazole) were defined as resistant

Table 2. Eradication rates for the triple therapy of lansoprazole-amoxicillin-metronidazole in patients who failed first-line therapy

	% (*n*)	95% CI
All patients	96.2 (51/53)	87.0–99.5
Clarithromicin resistant	97.5 (39/40)	
Metronidazole low sensitive[a]	100.0 (3/3)	

CI, confidence interval

[a] All the metronidazole-low sensitive strains were resistant to clarithromycin

patients who receive eradication therapy has been increasing. However, treatment failure has become an issue of concern. The acquisition of antibiotic resistance is the most common cause for eradication failure in many countries.[8,9] Consumption of antimicrobial agents increases resistant *H. pylori* strains before eradication therapy,[8] and the failure of eradication therapy is also associated with acquisition of antibiotic resistance.[10] Indeed, in our studies, the proportion of CLA-resistance was approximately 10% in patients who received first-line therapy,[11] but it increased to more than 80% in patients who had failed first-line therapy. At present, however, only AMOX and CLA are approved for the treatment of *H. pylori* infection in Japan, and resistance to CLA is easily acquired by *H. pylori*.[12] Although retreatment with first-line therapy for a period of 7–14 days was carried out after the failure of 1 week of this therapy, the eradication rate was not higher than that of the dual therapies of PPI and AMOX.[13] Because CLA-resistant strains have been increasing among Japanese *H. pylori* strains, the establishment of eradication therapy that does not contain CLA is required for patients who failed first-line therapy.

In Japan, the use of MNZ has been limited, and this may be associated with the lower frequency of MNZ-resistant strains in comparison with other Asian and Western countries.[4,5,14,15] However, in Japan, only a few studies have investigated the efficacy of MNZ for the treatment of *H. pylori* infection. Moreover, such studies had not been performed in patients who had failed first-line therapy until recently. In an earlier study, Miyaji et al.[16] demonstrated that triple therapy of PPI (one of OPZ and LPZ), AMOX, and MNZ eradicated *H. pylori* in 60 of 70 patients (85.7%). Recently, Murakami et al.[6] also showed a high eradication rate (88%) of triple therapy containing RPZ, AMOX, and MNZ in a western Japanese population after the failure of first-line therapy. However, treatment results of regimens containing MNZ could be affected by the frequency of MNZ resistance, and the existence of regional differences in MNZ resistance[7] has been reported in Japan. In our study, no MNZ-resistant strain was observed in 70 *H. pylori* isolates. Therefore, it is evident that the eradication rate of this study (96.2%) was higher than that shown by Murakami et al.[6] However, the eradication rate was almost the same as that in Murakami's study (97%) when patients infected with MNZ-resistant strains were excluded.[6] Furthermore, in the same study, the eradication rate was high (82%) even though the infected strain was determined as MNZ resistant in vitro. These results might indicate that, in some cases, resistance to MNZ affects the results of the triple therapy containing PPI, MNZ, and AMOX.

The high eradication rate observed in this study could be associated with the fact that MNZ-resistant strains were very rare in this northern region of Japan. Generally, in Japan, a higher prevalence of MNZ-resistant strains is seen in the western or southern regions. This difference would be explained by the close proximity of these regions to other Asian countries, which have a high prevalence of MNZ-resistant *H. pylori*. South and West Japan are much closer to these countries than North Japan. A number of people from other eastern Asian countries have lived in the Kansai and Kyushu areas. Furthermore, a larger number of people from western and southern Japan travel to these countries, where the hygienic conditions are not good. Therefore, it is easier for MNZ-resistant *H. pylori* to spread in southern or western Japan than in northern Japan. Additionally, among the areas in North Japan, Aomori Prefecture is recognized to have a low frequency of sexually transmitted diseases (STD) because prefectural regulations strictly limit business activities that are contrary to general moral values. Because the use of MNZ is limited to tricomoniasis, which is one of the most common STDs in Japan, use of MNZ is not frequent in Aomori Prefecture. Thus, in this northern Japanese region, the number of imported MNZ-resistant *H. pylori* is low and the opportunities to acquire the resistance to MNZ are also less frequent. Therefore, a high eradication rate was achieved by the triple therapy containing MNZ.

Suppression of gastric acid is relevant for the eradication therapy of *H. pylori* infection. PPI is recognized to stabilize AMOX in the gastric mucus, and eradication rates of regimens containing AMOX are affected by the dose of PPI.[17] It is well known that most PPIs are metabolized by CYP2C19. According to the metabolic activity, the genotype of CYP2C19 is divided into the extensive metabolizer and the poor metabolizer.[18] In some eradication regimens, higher eradication rates have been observed in poor metabolizers than in extensive metabolizers. On the other hand, the suppression of gastric acid secretion by PPIs reduces the transfer of MNZ into the gastric juice and decreases the concentration of MNZ in the gastric juice.[19,20] Therefore, the significance of PPIs for MNZ in the eradication of *H. pylori* has not been fully understood. Murakami et al.[6] used RPZ in their second-line therapy to avoid the influence of CYP2C19 status. In the present study, we used LPZ, which is mainly metabolized by CYP2C19, with the same doses of AMOX and MNZ that were used in Murakami's study, but a similar eradication rate was achieved. These results suggest that the genotyping of CYP2C19 is not necessary for the second-line triple therapy of PPI, AMOX, and MNZ, at least in patients who were infected with MNZ-sensitive *H. pylori*.

Another problem regarding this regimen is the limitation of the use of MNZ in Japan. Because the administration of MNZ has been associated with malignancies

such as lung cancer,[21] the Japanese system of health insurance has approved the use of MNZ only for tricomoniasis. However, the duration and the total amount of MNZ for the treatment of *H. pylori* infection are smaller than those approved for tricomoniasis. Thus, it is unlikely that the use of MNZ for the treatment of *H. pylori* infection would influence the risk of malignancies, even though evidence to support this claim is required.

In conclusion, in patients who failed first-line therapy, the triple therapy of LPZ, AMOX, and MNZ, achieved favorable eradication rates with few side effects. This regimen was effective in patients who were infected with CLA-resistant strains. Genotyping of CYP2C19 is not necessary, at least in regions where MNZ-resistant *H. pylori* infection is rarely observed. However, we cannot preclude the possibility that the eradication rate of this regimen is lower in patients infected with MNZ-resistant strains. MIC of MNZ should be studied before performing MNZ-containing regimens to understand the difference in treatment results across different Japanese regions.

Acknowledgments. The authors thank Dr. Nobuhiro Obara and the Aomori Clinical Study Group for *Helicobacter pylori* Infection for their contributions.

References

1. Satoh K. Indications for *Helicobacter pylori* eradication therapy and first-line therapy regimen in Japan: recommendation by the Japanese Society for *Helicobacter* Research. J Gastroenterol 2002;37(suppl 13):34–8.
2. Miwa H, Misawa H, Yamada T, Nagahara A, Ohtaka K, Sato N. Clarithromycin resistance, but not CYP2C-19 polymorphism, has a major impact on treatment success in 7-day treatment regimen for cure of *H. pylori* infection: a multiple logistic regression analysis. Dig Dis Sci 2001;46:2445–50.
3. Hoshiya S, Watanabe K, Tokunaga K, Tanaka A, Ninomiya H, Shingaki M, et al. Relationship between eradication therapy and clarithromycin-resistant *Helicobacter pylori* in Japan. J Gastroenterol 2000;35:10–4.
4. Hua JS, Bow H, Zheng PY, Khay-Guan Y. Prevalence of primary *Helicobacter pylori* resistance to metronidazole and clarithromycin in Singapore. World J Gastroenterol 2000;6:119–21.
5. Yakoob J, Fan X, Hu G, Liu L, Zhang Z. Antibiotic susceptibility of *Helicobacter pylori* in the Chinese population. J Gastroenterol Hepatol 2001;16:981–5.
6. Murakami K, Sato R, Okimoto T, Nasu M, Fujioka T, Kodama M, et al. Efficacy of triple therapy comprising rabeprazole, amoxicillin and metronidazole for second-line *Helicobacter pylori* eradication in Japan, and the influence of metronidazole resistance. Aliment Pharmacol Ther 2003;17:119–23.
7. Kato M, Yamaoka Y, Kim JJ, Reddy R, Asaka M, Kashima K, et al. Regional differences in metronidazole resistance and increasing clarithromycin resistance among *Helicobacter pylori* isolates from Japan. Antimicrob Agents Chemother 2000;44:2214–6.
8. Adamek RJ, Suerbaum S, Pfaffenbach B, Opferkuch W. Primary and acquired *Helicobacter pylori* resistance to clarithromycin, metronidazole, and amoxicillin: influence to treatment outcome. Am J Gastroenterol 1998;93:386–9.
9. Poon SK, Chang CS, Su J, Lai CH, Yang CC, Chen GH, et al. Primary resistance to antibiotics and its clinical impact on the efficacy of *Helicobacter pylori* lansoprazole-based triple therapies. Aliment Pharmacol Ther 2002;16:291–6.
10. Perez Aldana L, Kato M, Nakagawa S, Kawarasaki M, Nagasako T, Mizushima T, et al. The relationship between consumption of antimicrobial agents and the prevalence of primary *Helicobacter pylori* resistance. Helicobacter 2002;7:306–9.
11. Umegaki N, Shimoyama T, Nishiya D, Suto T, Fukuda S, Munakata A. Clarithromycin resistant and point mutation in the 23S rRNA gene in *Helicobacter pylori* isolates from Japan. J Gastroenterol Hepatol 2000;15:906–9.
12. Versalovic J, Shortridge D, Kibler K, Griffy MV, Beyer J, Flamm RK, et al. Mutations in 23S rRNA are associated with clarithromycin resistance in *Helicobacter pylori*. Antimicrob Agents Chemother 1996;40:477–80.
13. Nagahara A, Miwa H, Ohkura R, Yamada T, Sato K, Hojo M, et al. Strategy for retreatment of therapeutic failure of eradication of *Helicobacter pylori* infection. J Gastroenterol Hepatol 2001;16:613–8.
14. Prazeres Magalhaes P, De Magalhaes Queiroz DM, Campos Barbosa DV, Aguiar Rocha G, Nogueira Mendes E, Santos A, et al. *Helicobacter pylori* primary resistance to metronidazole and clarithromycin in Brazil. Antimicrob Agents Chemother 2002;46:2021–3.
15. Megraud F. Epidemiology and mechanism of antibiotic resistance in *Helicobacter pylori*. Gastroenterology 1998;115:1278–82.
16. Miyaji H, Azuma T, Ito S, Suto H, Ito Y, Yamazaki Y, et al. Susceptibility of *Helicobacter pylori* isolates to metronidazole, clarithromycin and amoxycillin in vitro and in clinical treatment in Japan. Aliment Pharmacol Ther 1997;11:1131–6.
17. Kawano S, Murakami M, Saita H, Tsuji S. Effect of lansoprazole in mono-, dual-, or triple therapy on *Helicobacter pylori* eradication. J Gastroenterol 1996;31(suppl 9):41–3.
18. Ishizaki T, Horai Y. Cytochrome P450 and the metabolism of proton pump inhibitors: emphasis on rabeprazole. Aliment Pharmacol Ther 1999;13(suppl 3):27–36.
19. Goddard AF, Jessa MJ, Barrett DA, Shaw PN, Idstrom JP, Cederberg C, et al. Effect of omeprazole on the distribution of metronidazole, amoxicillin, and clarithromycin in human gastric juice. Gastroenterology 1996;111:358–67.
20. Sherwood PV, Wibawa JI, Atherton JC, Jordan N, Jenkins D, Barrett DA, et al. Impact of acid secretion, gastritis, and mucus thickness on gastric transfer of antibiotics in rats. Gut 2002;51:490–5.
21. Beard CM, Noller KL, O'Fallon WM, Kurland LT, Dahlin DC. Cancer after exposure to metronidazole. Mayo Clin Proc 1988;63:147–53.